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EFFECT OF ACHENE MORPHOLOGY AND MASS ON GERMINATION AND SEEDLING GROWTH OF *BOLTONIA DECURRENS* (ASTERACEAE), A THREATENED FLOODPLAIN SPECIES

MARIAN SMITH

Department of Biological Sciences, Southern Illinois University,
Edwardsville, IL 62026-1651
e-mail: msmith@siue.edu

JOHN CAWLY

Department of Plant Microbiology and Pathology, University of Missouri,
Columbia, MO 65211-6130
e-mail: jdc3d9@missou.edu

ABSTRACT. *Boltonia decurrens* (Asteraceae), a plant species endemic to the Illinois River Valley, is threatened with extinction. Alterations of the hydrologic regime of the river have resulted in habitat loss and population decline. Lack of information about the complex life cycle of this species frustrates efforts to develop an effective recovery plan. An essential part of any recovery plan is an understanding of seed germination, seedling recruitment, and early growth, and how each contributes to the maintenance of a population. In *B. decurrens*, the dimorphic achenes have different masses and may provide different dispersal mechanisms. This study examined the effect of achene morphology and mass on seed germination and early seedling growth of *B. decurrens* under controlled environmental conditions. There was no difference in timing of germination of disk and ray achenes in *B. decurrens*; however, there was a distinct difference in early growth of seedlings derived from disk versus ray achenes and from larger ray achenes compared to smaller ones. Disk achenes, which produced seedlings with more leaf area during the first 10–15 days, may provide a competitive advantage over those produced by ray achenes. Since leaf area and photosynthetic rates are closely correlated, seedlings with more leaf area early in their development may also be more competitive with seedlings of other species.

Key Words: dimorphic seeds, germination, seedling growth, seed size, threatened species, floodplain, *Boltonia*

Boltonia decurrens (Torr. & A. Gray) A. Wood (Asteraceae) is an herbaceous perennial whose distribution is restricted to the Illinois River floodplain (Torrey and Gray 1841; U.S. Fish and Wildlife Service 1990). The species is on the Federal List of threatened species (U.S. Fish and Wildlife Service 1988), and is currently listed as a Species of Concern in Missouri (Missouri

Department of Conservation 1999) and as Threatened in Illinois (Herkert 1991). Inflorescences are borne on panicles and produce prolific numbers of dimorphic achenes (Smith and Keevin 1998). Additionally, vegetative ramets that overwinter and reproduce sexually the following year are produced at the base of the senescing flowering plants each fall (Redmond 1993; Smith 1991; Smith et al. 1998). Despite these reproductive strategies, the construction of levees and navigation dams along the Illinois River have resulted in habitat loss and a decline in population size and number (Schwegman and Nyboer 1985; Smith et al. 1998; U.S. Fish and Wildlife Service 1990).

Dissimilarities in seed morphology within plant taxa may contribute to different dispersal and germination patterns as well as different growth and fitness of the resulting plants (Banovetz and Scheiner 1992; Zhang 1993). Disk flowers of *Boltonia decurrens* produce flattened, heart-shaped achenes that are characterized by a pappus of two bristles. Disk achenes average 1.8 mm in length and 1.3 mm in width with an average mass of 0.1 mg (Smith and Keevin 1998). Achenes produced from ray flowers are smaller and wedge-shaped, possessing a distinct third side, and they have greatly reduced bristles. Ray achenes average 1.3 mm in length and 0.9 mm in width, and they average 0.05 mg in mass (Smith and Keevin 1998). The increased surface area/mass ratio of the disk achenes provides greater buoyancy and allows them to float for extended periods of time (> 30 days; Smith and Keevin 1998), and this may facilitate the establishment of remote populations after flood waters recede. The wedge-shaped ray achenes do not float and may contribute to the maintenance of the species at or near an extant population (Smith and Keevin 1998; Smith et al. 1998).

Germination and early seedling development are the most critical stages in the life cycle of a plant (Harper et al. 1970), and any variation in achene morphology or mass that could affect germination or early growth could also influence the establishment of new populations or the maintenance of extant populations. Although germination studies have been conducted on *Boltonia decurrens* (Baskin and Baskin 1988; Smith and Keevin 1998; Smith et al. 1995), little is known of germination patterns or seedling growth specific to achene morphology or mass. The present study examines the effect of achene morphology and mass on germination and early seedling growth.

MATERIALS AND METHODS

Achenes were collected from West Alton, St. Charles County, Missouri (lat. 38°52′06″N, long. 90°12′22″W) and maintained at 5°C for two years before the initiation of this study. Size classes of each achene morphology were differentiated by sorting through a series of screens with mesh sizes of 0.841 mm, 0.595 mm, and 0.420 mm, followed by manual segregation of each morphological type using a stereoscope. The largest size class ($>$ 0.841 mm) contained predominantly disk achenes (D1); the smallest ($>$ 0.420 mm but $<$ 0.595 mm) contained mature ray achenes (R3) and a very small proportion of immature or non-viable disk achenes (lacking a visible embryo). Both mature disk and ray achenes (D2 and R2) were represented in the intermediate size class ($>$ 0.595 mm but $<$ 0.841 mm). Seeds with evidence of herbivory, or apparently nonviable (no visible embryo), were discarded. Otherwise, seeds were selected randomly to minimize possible sampling bias. Each of the ten replicates consisted of 25 disk and 25 ray achenes in each of two size classes, for a total of 1000 achenes. Dry mass was recorded for each sample of 25 seeds, and a mass : size correlation was calculated.

Since the achenes require light for germination (Baskin and Baskin 1988; Smith and Keevin 1998), they were germinated in 10 cm square pots on the surface of a commercial, peat-based growing medium in a Sherer CEL-25 7HL environmental chamber at 20°C and 200 μmol m^{-2} s^{-1} PPF (photosynthetic photon flux), which was measured using an LI-185B quantum meter and LI-190SB quantum sensor (LI-COR, Lincoln, NE). Germination was determined by radicle emergence, recorded daily, and seedlings were identified with a color-coded pin. Length and width measures of the cotyledons were taken after five days of growth. Measures were obtained for both cotyledons and true leaves at 10 and 15 days. Cotyledons and primary leaves are not lobed, do not have serrated edges, and are approximately rectangular; therefore, leaf area was calculated using the algorithm for area of a rectangle (L $\times$ W). After 15 days the seedlings were transferred to a greenhouse, where they were initially placed under 50% shade cloth to minimize photodamage and were exposed to higher ambient light levels over a two-week period. Light levels were measured in the greenhouse at 12 noon daily during the study (June and July) and averaged 1500 $\pm$ 350 μmol m^{-2} s^{-1} PPF. Pots were watered daily and rotated to minimize environmental variation due to position. Seedling height was measured at 30 days and at 60 days. Because *Boltonia decurrens* is endangered, plants were not harvested for biomass measurements, but were transplanted into the population from which the seeds had been collected.

All analyses were performed using SYSTAT 5.2 (SPSS, Chicago, Illinois). The achene size class and mass relationship was determined using linear regression, and germination and seedling survival data were analyzed using a chi square statistic of a contingency table (Steel and Torrie 1980). For the χ^2 analyses of germination and survival, data from replicates were pooled within each achene class.

The unequally represented size classes, which resulted from differences in the numbers of seeds that germinated within each achene class, produced a non-orthogonal design that would affect the relationship of the other classes in calculating the F-ratio (Steel and Torrie 1980); therefore, we used Multivariable General Linear Hypothesis (MGLH; type III sum of squares) for all analyses of variance (ANOVAs) of leaf area and height. Log transformation was used to normalize leaf area and height data (Steel and Torrie 1980). Linear contrast analyses were used for pairwise comparisons of seedling leaf area and seedling height for each size class and morphology combination in accordance with Steel and Torrie (1980).

RESULTS

Germination of achenes and seedling survival. There was no significant difference in germination among disk and ray achenes of any class (χ^2 = 2.751; P = 0.432; df = 3; Figure 1). Of the 1000 seeds used in this experiment, 667 germinated: 339 disk achenes and 328 ray achenes. Although there was no statistical difference in survival among achene classes (χ^2 = 2.570; P = 0.497; df = 3), there was a trend for a decrease in survival with a decrease in achene size (Figure 1). Disk and ray achenes of all classes demonstrated the same germination pattern (Figure 2): germination peaked on day 4, with no germination occurring before day 3 or after day 11. There was a positive linear relationship (r^2 = 0.8165; $P < 0.05$; df = 3) between achene size (area) and mass (i.e., the larger the achene, the greater the mass).

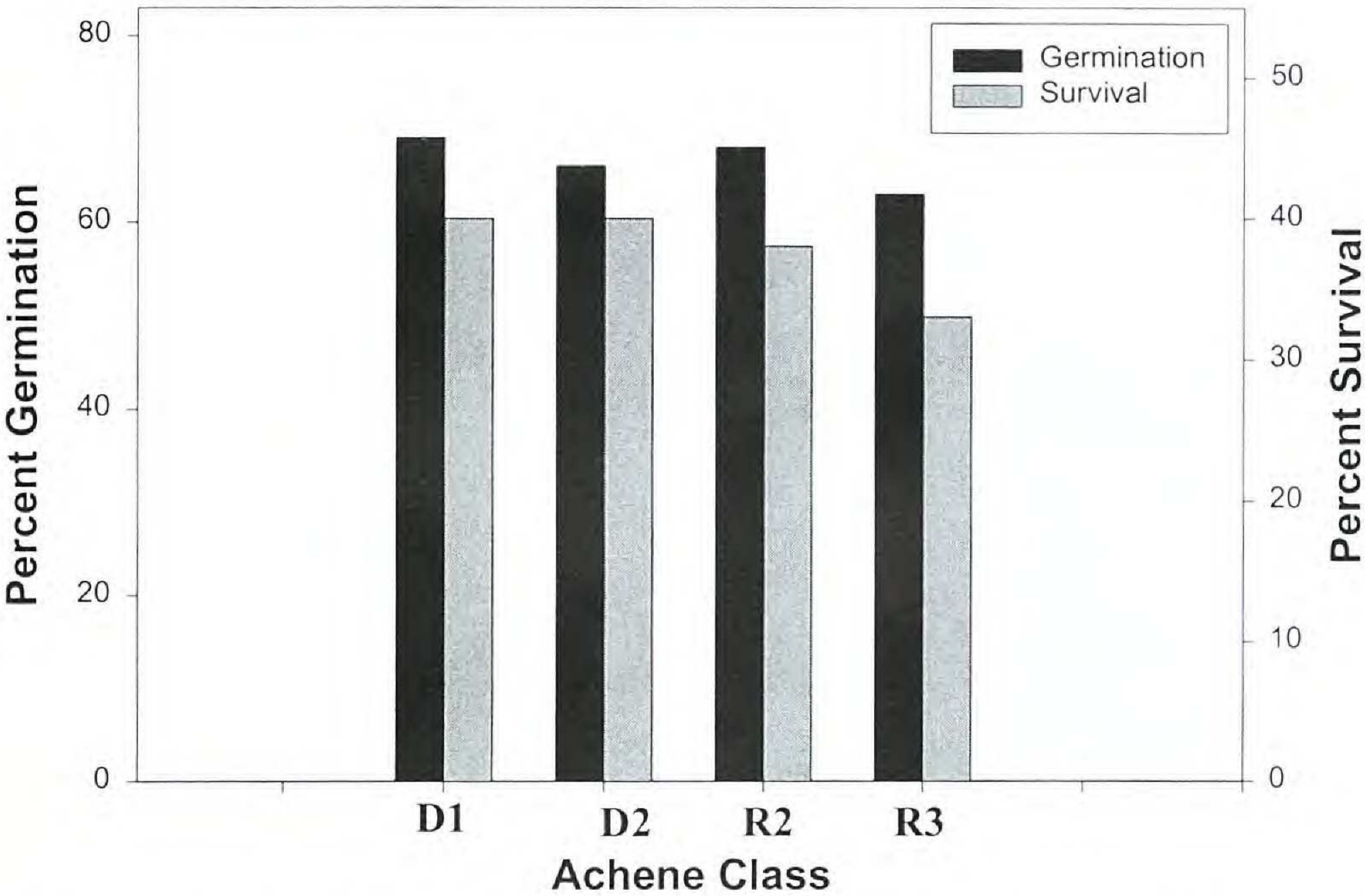

Figure 1. Percent germination and percent seedling survival for achenes from all size classes. D1 = disk achene size class 1 (> 0.841 mm). D2 = disk achene size class 2 (> 0.420 mm, but < 0.841 mm). R2 = ray achene size class 2 (> 0.420 mm, but < 0.841 mm). R3 = ray achene size class 3 (< 0.420 mm).

Seedling growth. One-way analysis of variance (ANOVA) of leaf area measurements taken after 10 days of growth indicated a statistical significance (Table 1). Linear contrast analysis comparing leaf areas of seedlings from both seed types in all size classes indicated statistical significance when contrasting all disk achenes versus all ray achenes (Table 2; Figure 3), the two size classes of ray achenes (R2 and R3), and the largest size class (D1) versus the smallest class (R3; Table 2; Figure 4).

Similarly, ANOVA of 15-day measurements showed statistical difference (Table 1), and linear contrast analysis exhibited statistical significance in comparing all disk achenes versus all ray achenes (Table 2; Figure 3). Additionally, at 15 days, the larger class of disk achenes (D1) proved to have significantly greater leaf area when compared with all other size classes (Table 2; Figure 4). No statistical significance was found by ANOVA of plant height measurements taken after 30 days or after 60 days of growth; furthermore, linear contrast analysis revealed no statistically significant differences in any size class or morphology comparison at 30-day or 60-day measurements. There is, how-

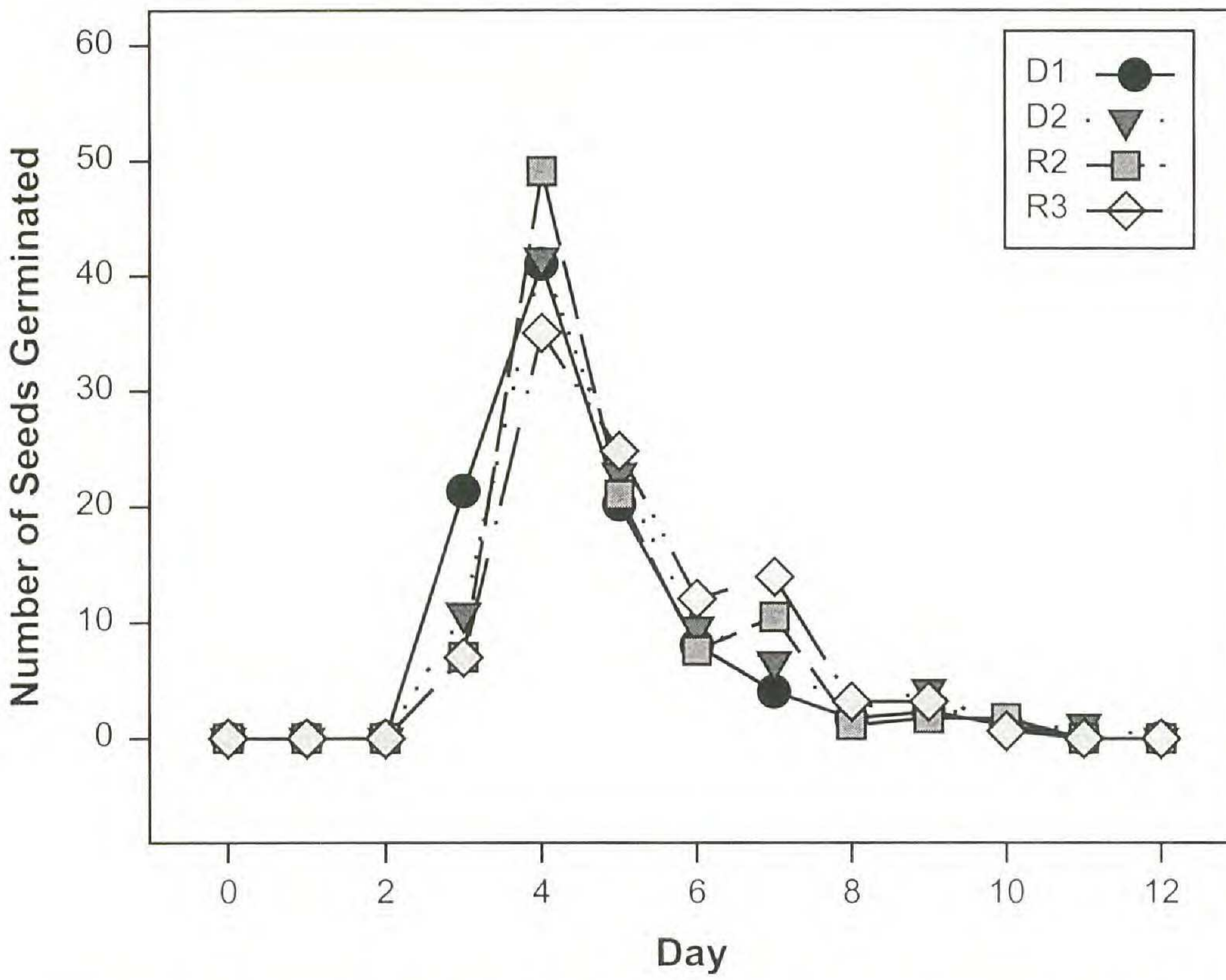

Figure 2. Number of achenes of *Boltonia decurrens* germinating at 22°C and 200 μmol m^{-2} s^{-1} PPF over a 12-day period. D1 = disk achene size class 1 (> 0.841 mm). D2 = disk achene size class 2 (> 0.420 mm, but < 0.841 mm). R2 = ray achene size class 2 (> 0.420 mm, but < 0.841 mm). R3 = ray achene size class 3 (< 0.420 mm).

Table 1. Results of the one-way ANOVAs for seedling leaf area at 10 and 15 days, and seedling height at 30 and 60 days among all four achene classes.

	Source	SSE	df	MSE	F	*P*
10 days	Type	1.923	3	0.641	4.498	0.004
	Error	28.517	200	0.143		
15 days	Type	1.499	3	0.500	4.420	0.005
	Error	16.501	146	0.113		
30 days	Type	0.313	3	0.104	1.358	0.256
	Error	19.273	251	0.077		
60 days	Type	0.196	3	0.128	1.253	0.291
	Error	25.709	251	0.102		

Table 2. Linear contrast analyses for seedling leaf area and seedling height. D1 = disk achene size class 1 (> 0.841 mm). D2 = disk achene size class 2 (> 0.420 mm, < 0.841 mm). R2 = ray achene size class 2 (> 0.420 mm, < 0.841 mm). R3 = ray achene size class 3 (< 0.420 mm).

	Disk vs. Ray		D1 vs. D2		D1 vs. R2		D1 vs. R3		D2 vs. R2		D2 vs. R3		R2 vs. R3	
Contrasts	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
10 days	8.461	0.004	0.067	0.796	1.308	0.254	10.842	0.001	0.768	0.382	9.079	0.003	4.689	0.038
15 days	4.870	0.029	7.684	0.006	6.618	0.011	11.173	0.001	0.039	0.843	0.301	0.584	0.559	0.456
30 days	2.277	0.133	1.770	0.185	3.023	0.083	2.821	0.094	0.174	0.677	0.194	0.660	0.002	0.961
60 days	1.910	0.168	1.816	0.179	2.400	0.123	2.887	0.091	0.045	0.833	0.197	0.658	0.060	0.807

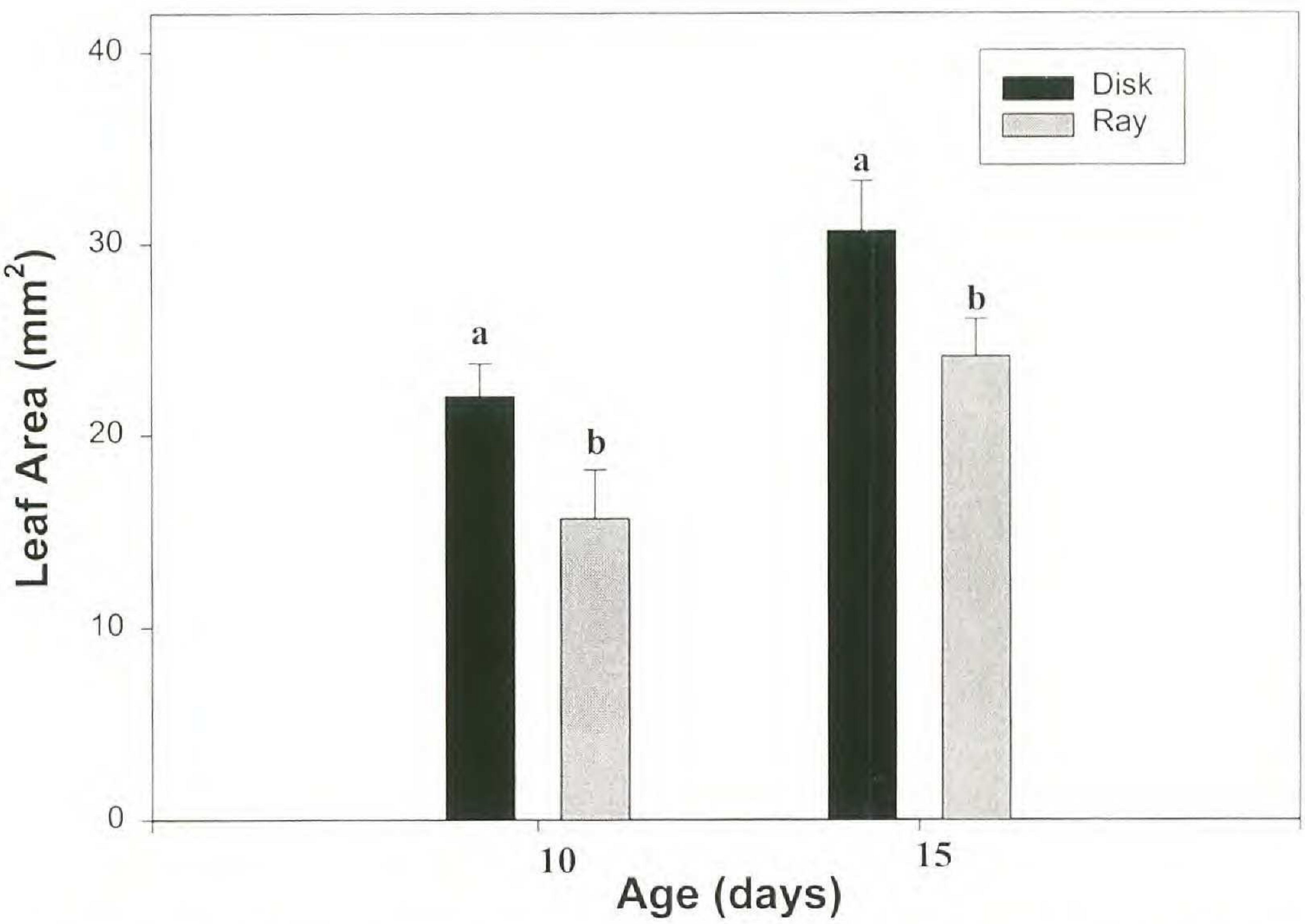

Figure 3. Mean leaf area (± SE) produced by disk and ray achenes, regardless of size class, after 10 and 15 days of growth. Bars with different letters are significantly different between achene types (see Table 2 for *P* values).

ever, an indication that height in the plants produced from the smallest size class, R3, began to lag behind the others in growth at 60 days (Figure 5).

DISCUSSION

Our results suggest that seed morphology influences seedling establishment during the early stages of development; however, any differential competitive advantage due to achene type or mass is less obvious after 10–15 days of growth. Grime (2001) proposed that differences in seed morphology may influence seedling establishment and growth, and our results with *Boltonia decurrens* appear to corroborate his findings. Total leaf area after 10–15 days of growth differed significantly between disk and ray achenes. Additionally, comparison of 10-day growth was significant by size class (i.e., larger achenes produced seedlings with greater leaf area). Since the photosynthetic area of the cotyledon, rather than its mass or stored energy, is the primary consideration in early seedling development (Harper et al. 1970), achenes pro-

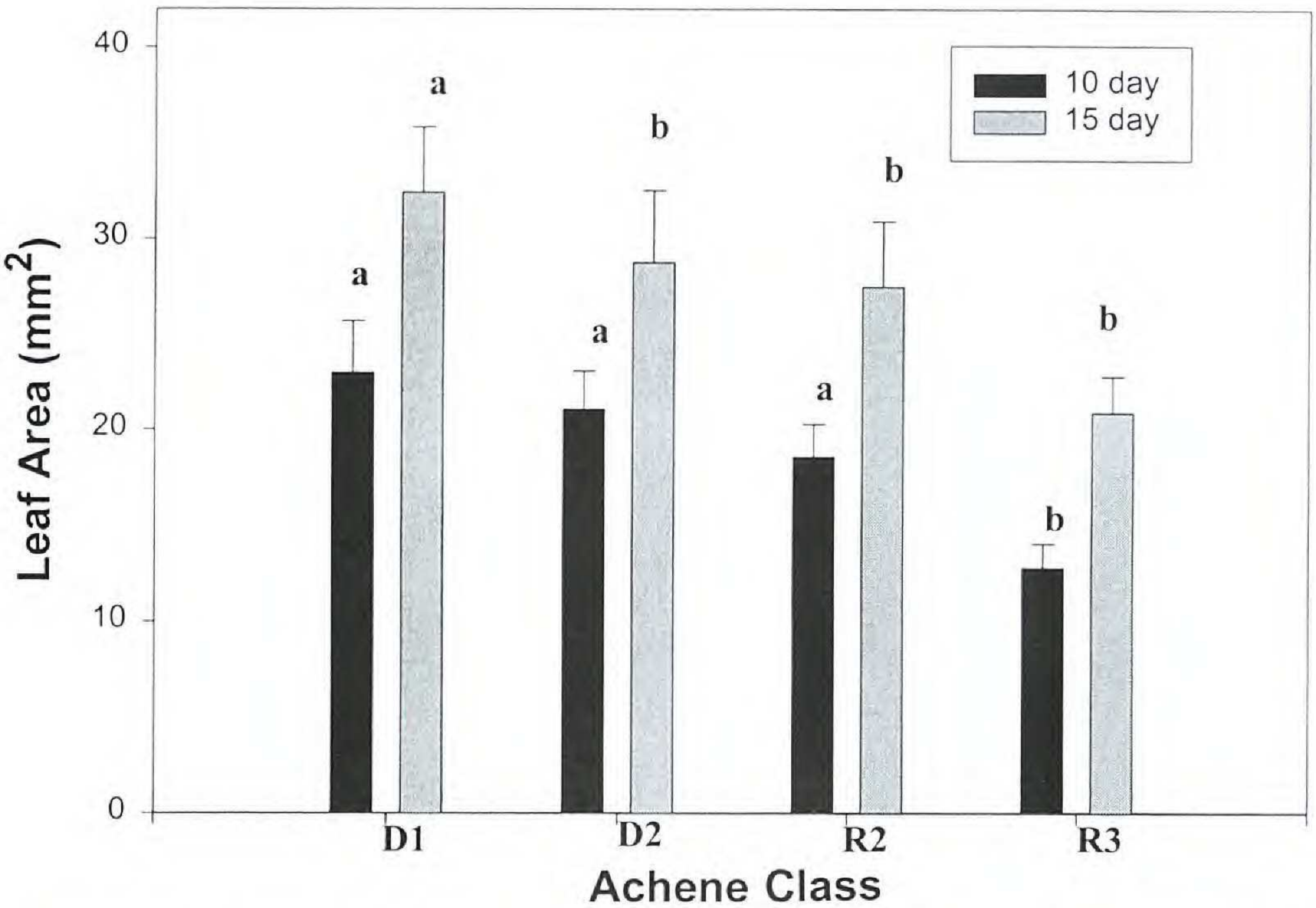

Figure 4. Mean leaf area (± SE) in each achene class after 10 and 15 days of growth. D1 = disk achene size class 1 (> 0.841 mm). D2 = disk achene size class 2 (> 0.420 mm, but < 0.841 mm). R2 = ray achene size class 2 (> 0.420 mm, but < 0.841 mm). R3 = ray achene size class 3 (< 0.420 mm). Bars with different letters are significantly different for each comparison between achene classes (see Table 2 for *P* values).

ducing small cotyledons would have relatively less potential for growth than those possessing large cotyledons. After 15 days of growth, the advantage of greater cotyledon area is reinforced by the greater total leaf area (cotyledons plus true leaves).

Boltonia decurrens requires light for germination (Baskin and Baskin 1988; Smith and Keevin 1998) and high light for growth and seed production (Smith et al. 1993), and seedlings have high mortality when germinated under plant litter (Smith et al. 1995). In natural populations, seedling establishment is extremely low after one year of succession (< 0.01%; Moss 1997; Smith et al. 1998), and *B. decurrens* is often completely replaced by competing vegetation 3–5 years after population establishment (Schwegman and Nyboer 1985; U.S. Fish and Wildlife Service 1990). If achene morphology or mass determines cotyledon and leaf size for the first 10–15 days, greater photosynthetic surface area during this period may enable these seedlings to be more competitive with rapidly growing seedlings of other species. Data

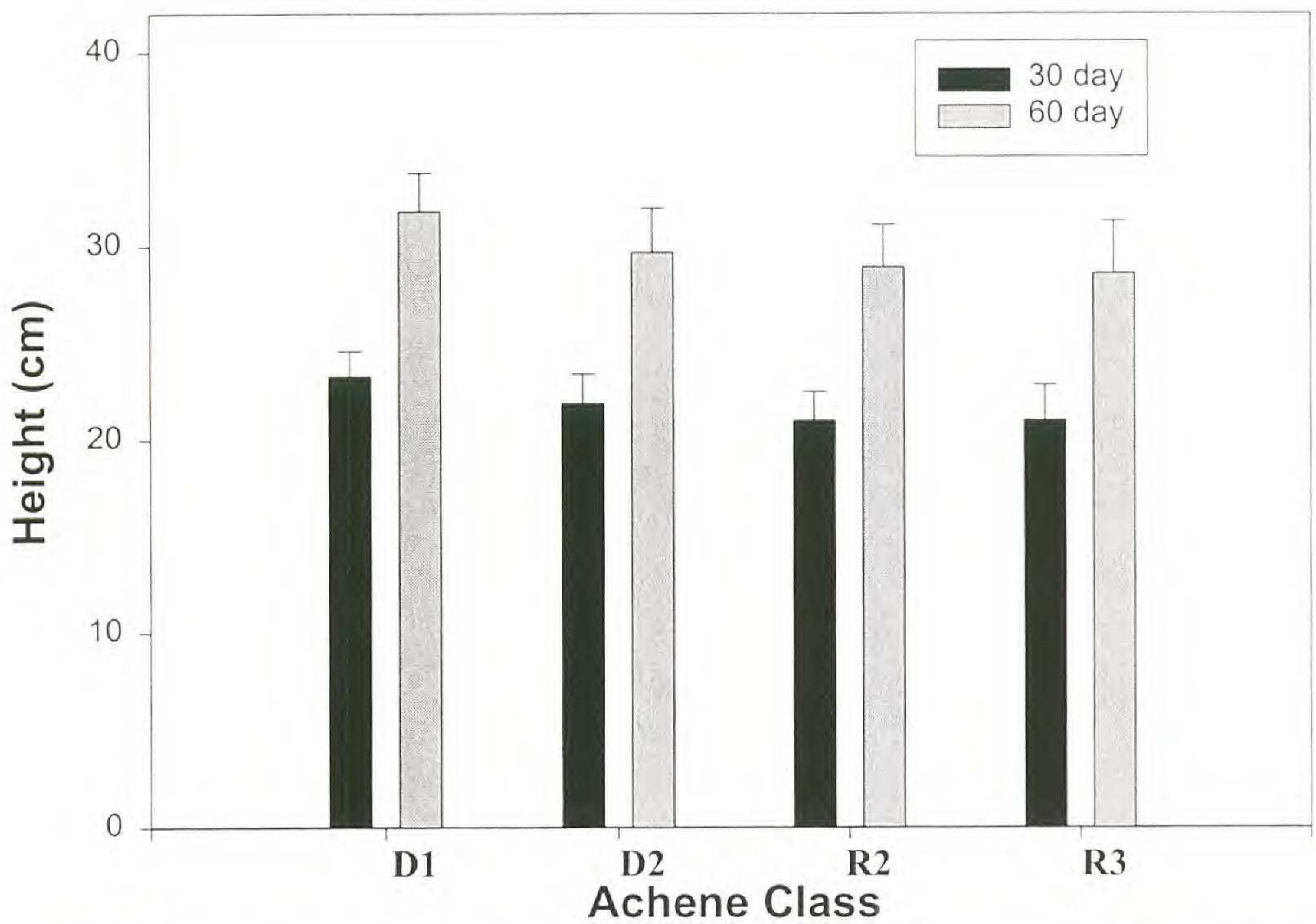

Figure 5. Mean seedling height (± SE) in each achene class after 30 and 60 days of growth. D1 = disk achene size class 1 (> 0.841 mm). D2 = disk achene size class 2 (> 0.420 mm, but < 0.841 mm). R2 = ray achene size class 2 (> 0.420 mm, but < 0.841 mm). R3 = ray achene size class 3 (< 0.420 mm).

from our study indicate that seedlings from disk achenes, regardless of size, would have the highest probability of surviving, and that seedlings from larger ray achenes would fare better than those from smaller ones.

Although there may have been height or mortality differences among seed morphologies and sizes during the earliest stages of growth, at 30 and 60 days there were no statistically significant differences in either. In the present study, the widely spaced distribution of seedlings across the soil surface minimized interactions between individuals. Similar conditions do not exist in the field, however, where thousands of seeds germinate simultaneously. This is particularly so in the case of *Boltonia decurrens*, because its achenes and the seeds of other species are often deposited in densely packed rows by receding floodwaters (Smith and Keevin 1998; Smith et al. 1995). In this environment, where resources are limited and competition increases seedling mortality, a 10–15 day advantage provided by greater photosynthetic surface area may be critical for seedling growth and survival.

In other Asteraceae, Fenner (1983) found that performance of seedlings in the period immediately after germination is critical for establishment. Although Fenner's study was not designed to be a competition experiment, or to represent a field situation, some important inferences can be drawn from his data. Clearly, chronological differences in seedling emergence affect competitive interactions among seedlings: those emerging earlier potentially shade later-germinating seedlings and inhibit their growth. That this effect is largely due to differential seedling size is supported by the results of Gross (1984), who found that within-species differences in seed size had a significant effect on early seedling growth and survival. Although both types of achenes of *Boltonia decurrens* have similar temporal patterns of germination, size differences due to achene morphology or mass produce the same result—larger seedlings that are less likely to be overtopped by other seedlings. In *B. decurrens,* this is particularly important due to its requirement for high light during all stages of growth.

A competitive advantage during early seedling development may be essential to the survival of *Boltonia decurrens* in its current habitat. The construction of a series of levees and navigation dams on the Illinois River over the past 70 years has altered the natural flood regime of the Illinois River Valley (Sparks 1995; Sparks et al. 1998). Areas that once provided the open moist shorelines suitable for the establishment and regeneration of populations of *B. decurrens* are now seldom flooded, resulting in the invasion of the sites by a number of aggressive species that are less flood tolerant than *B. decurrens* (Schwegman and Nyboer 1985; Smith 1991; Smith et al. 1998). In these areas, individuals of *B. decurrens* become smaller and produce fewer and smaller achenes each year following population establishment (Smith 1993). Seedling survival declines rapidly (Moss 1997) as the number and density of aggressive competitors increase. Recent work by Mettler et al. (2001) and Smith (unpubl. data) indicates that the loss of an annual nutrient pulse in years without floods may contribute to the decline of *B. decurrens* by reducing plant size and achene number and mass.

Information provided in the current study indicates that a reduction in achene size would affect seedling growth, and adds to the accumulating evidence that alterations in the natural flood regime in the Illinois River Valley are implicated in the decline of *Boltonia decurrens* (Smith and Mettler 2002; Smith et al.

1998), as has been reported for other native species (Sparks 1995; Sparks et al. 1998). This information may help stimulate efforts to restore connections between the river and the floodplain and to re-establish native plant communities in the Illinois River Valley.

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